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# SHORT REPORT: HIGH INCIDENCE OF SHIGELLOSIS AMONG PERUVIAN SOLDIERS DEPLOYED IN THE AMAZON RIVER BASIN

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*Abstract.* We investigated the etiology of acute diarrhea among Peruvian military recruits undergoing three months of basic combat training near the Amazonian city of Iquitos. From January through September 2002, 307 of 967 recruits were seen at the Health Post for diarrhea (attack rate [AR] = 31.8%, incidence = 1.28 95% confidence interval [CI] = 1.14–1.43] episodes/person-year). *Shigella* spp. were the most common bacterial pathogen recovered from recruits experiencing diarrhea episodes. These bacteria were isolated from 89 (40%) of 225 diarrheal stools examined (AR = 7.6%, incidence = 0.30 [95% CI = 0.24–0.38] episodes/person-year). Most (83 of 90; 92%) of the *Shigella* isolates were *S. flexneri*, of which 57 (69%) were serotype 2a. Seventy-six percent of *Shigella* isolates were resistant to sulfamethoxazole/trimethoprim and all were sensitive to ciprofloxacin. Peruvian soldiers may be an excellent population in which to test the efficacy of *S. flexneri* vaccines in advanced development.

Traveler's diarrhea (TD) constitutes the most common illness affecting international travelers[1] and strikes between 20% and 60% of short-term travelers to Africa or Latin America. The illness interrupts the travel plans of more than half of these travelers, and approximately one-third end up confined to bed.[2,3] Nausea, vomiting, fever, malaise, myalgias, abdominal cramping, and frequent watery stools account for the acute debilitating nature of TD. Enterotoxigenic *Escherichia coli* (ETEC), *Campylobacter* spp., and *Shigella* spp. are estimated to cause approximately 17–70%, 1–5%, and 2–30%, respectively, of diarrhea cases among travelers to Latin America.[1,4] Traveler's diarrhea also creates significant problems for deployed military troops. Rates as high as 43% (1–43%) have been documented among U.S. troops deployed to Latin America in short-term missions of 2–8 weeks.[5] Military populations seem especially vulnerable to TD, probably reflecting high risk practices, such as consumption of local fruits, raw vegetables, poorly cooked meat, non-potable water, and ice.[6,7] Only a few published studies, primarily from Israel, have documented the overall and agent-specific incidence and severity of acute diarrheal disease (ADD) among indigenous military personnel in a highly endemic area.[8,9] The work described here examines the cause and severity of ADD among Peruvian military recruits undergoing combat training in the Amazon jungle of eastern Peru, near the city of Iquitos.

Surveillance of ADD was established from January 1, 2002 through September 30, 2002 at one of the largest military training facilities in Peru, the Centro de Instruccion de Reclutas, 5th Military Region (5th CIR), on the army base Vargas-Guerra Ejercito (VGE). Patients were Peruvian military recruits (males 18–26 years old) assigned to a three-month cycle of basic combat training. Those who visited the health post at VGE with ADD, defined by three or more loose or watery stools or two loose or watery stools accompanied by nausea, vomiting, abdominal cramps/pain, or tenesmus within any 24-hour period, were asked to provide a stool sample in Carey-Blair transport medium. The sample was transported to Naval Medical Research Center Detachment–Iquitos for culture[10–12] and sensitivity testing against 12 antibiotics[13] (Table 1). Five lactose-fermenting colonies morphologically typical of *E. coli* were tested for the presence of ETEC heat-labile and heat-stable enterotoxin gene sequences by polymerase chain reaction (PCR).[14]

A total of 967 recruits trained in three periods, January–March (summer; n = 464 for 114 person-years), April–June (fall; n = 232 for 58 person-years), and July–September (winter; n = 271 for 68 person-years), for a cumulative 240 person-years at risk (Table 2). Of these, 307 reported to the Health Post with ADD (overall attack rate [AR] = 31.8%, incidence = 1.28 episodes per person-year; Table 2). The AR of diarrhea was significantly higher in the fall and winter recruiting classes than in the summer class ($P < 0.001$; Table 2). Little temperature variation is observed in Iquitos across seasons, but the fall and winter months correspond to the dry season and an increased incidence of ADD. No statistical correlation, however, was found between rainfall or Amazon River level and ADD incidence.

Of the 307 recruits with diarrhea, stool samples were obtained from 225 (73%). At least one bacterial enteropathogen was isolated from 118 (52%) of 225 samples and two enteropathogens were isolated in 18 of the 118 positive stools. *Shigella* spp. were the most common enteropathogens isolated; 89 (40%) of 225 samples were positive. In one of these samples, two *Shigella* serogroups were identified, for a total of 90 *Shigella* spp. isolates. Based on 73 (82%) cases of ADD in which *Shigella* was the only pathogen isolated, the overall AR of shigellosis was estimated to be 7.6% (95% confidence interval [CI] = 7.1–8.1%, by Life table analysis) with an incidence of 300 episodes of shigellosis per 1,000 person-years (Table 2). Although no statistical difference between seasons was found for *Shigella*-specific ARs, *Shigella* spp. were isolated more frequently between February and March ($P <$ 0.005, by Ratcher circular scan test). Eighty-three (92%) of the 90 *Shigella* spp. isolates were *S. flexneri* (66 were single infections) and seven (7.8%; all single infections) were *S. boydii.* The majority (n = 57; 69%) of *S. flexneri* isolates were serotype 2a, while 25% (n = 21) were serotype 1b.

Sixteen *Shigella*-positive stools were co-infections with *Plesiomonas shigelloides* (n = 7), *Campylobacter* spp. (n = 6), or *Aeromonas* spp. (n = 3). Enterotoxigenic *E. coli* was mixed with *A. hydrophila* in one case of ADD. Other enteropathogens isolated as single infections included *Campylobacter* spp. (n = 8; 3.6%), *P. shigelloides* (n = 6; 2.7%), *Aeromonas* spp.

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TABLE 1
Antimicrobial resistance of bacterial pathogens from diarrheic patients*

| Organisms (no. of isolates) | % of isolates resistant to the following antimicrobials | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amx | Am | Az | Cro | C | Cf | Cip | E | G | Nal | Sxt | Tet |
| *Shigella* (90) | 7.8 | 92 | 2.2 | 0 | 89 | 2.2 | 0 | 92 | 0 | 1.1 | 76 | 97 |
| *Aeromonas* (7) | 57 | 71 | 14 | 0 | 43 | 0 | 0 | 71 | 0 | 43 | 43 | 57 |
| *Campylobacter* (14) | 0 | 0 | 0 | 0 | 0 | 86 | 0 | 7.1 | 0 | 7.1 | 64 | 0 |
| ETEC (6) | 16 | 33 | 17 | 0 | 17 | 33 | 16 | 50 | 17 | 17 | 33 | 17 |
| *Plesiomonas shigelloides* (13) | 0 | 69 | 0 | 0 | 0 | 0 | 0 | 54 | 46 | 31 | 15 | 0 |
| *Salmonella typhi* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Amx = amoxacillin/clavulanate; Am = ampicillin; Az = azithromycin; Cro = ceftriaxone; C = chloramphenicol; Cf = cephalothin; Cip = ciprofloxacin; E = erythromycin; G = gentamicin; Nal = naladixic acid; Sxt = sulfamethoxazole/trimethoprim; Tet = tetracycline; ETEC = enterotoxigenic *Escherichia coli.*

(n = 3; 1.3%), ETEC (n = 5; 2.2%), and *Salmonella typhi* (n = 1; 0.44%). *Campylobacter* and *Aeromonas* species included *C. coli* (n = 5), *C. jejuni* (n = 2), *Campylobacter* sp. (n = 1), *A. caviae* (n = 1), and *A. hydrophila* (n = 2). Five strains were biochemically identified as *Shigella* spp. but did not agglutinate with typing serum. These underwent further analysis; two were PCR positive for the gene encoding invasion plasmid antigen H (*ipaH*),[15] indicating that they might be strains of enteroinvasive *E. coli* or strains of *Shigella* not represented by the antiserum panel used, and three were unconfirmed.

Many of the isolates were resistant to multiple antimicrobials (Table 1). *Shigella* spp. exhibited the highest overall level of resistance to antibiotics used most commonly in the past to treat diarrhea, particularly in travelers: tetracycline (97%), ampicillin (92%), and sulfamethoxazole/trimethoprim (76%). None of the isolates, except for one strain of ETEC, were resistant to ciprofloxacin. The absence of ciprofloxacin-resistance may reflect the under-prescribing of this antimicrobial and other fluroquinolones (Escobedo J, unpublished data) at VGE.

Patients were queried about symptoms and impact of their disease on daily routines. The following scale was adopted: minimal impact = no change in daily plans; moderate impact = alteration in daily activities precipitated; major impact = interruption in daily activities, e.g., stay at home or not do work; and severe impact = patients seek medical attention and would be hospitalized. Abdominal cramps was the most common complaint, reported by 119 (53%) of 223 patients. Thirty-eight percent of patients (85 of 225) reported nausea. The majority (65%; 143 of 221) reported diarrhea as moderately affecting their day, while 34% (76 of 221) reported major or severe impact upon their routines.

TABLE 2
Attack rate and incidence of diarrhea and shigellosis by recruit class

| | Episodes | AR (95% CI)* | ID (95% CI)† |
|---|---|---|---|
| Diarrhea | | | |
| Jan–Mar | 96 | 20.7% (17.1–24.7) | 0.84 (0.68–1.03) |
| Apr–Jun | 94 | 40.5% (34.1–47.1)‡ | 1.63 (1.31–1.99)§ |
| Jul–Sep | 117 | 43.2% (37.2–49.3)‡ | 1.71 (1.42–2.05)§ |
| Overall period | 307 | 31.8% (28.8–34.8) | 1.28 (1.14–1.43) |
| Shigellosis | | | |
| Jan–Mar | 29 | 6.3% (4.2–8.9) | 0.25 (0.17–0.36) |
| Apr–Jun | 23 | 9.9% (6.4–14.5) | 0.40 (0.25–0.60) |
| Jul–Sep | 21 | 7.8% (4.9–11.6) | 0.31 (0.19–0.47) |
| Overall period | 73 | 7.6% (6.0–9.4) | 0.30 (0.24–0.38) |

* AR = attack rate: episodes per number of recruits per period × 100 (the 95% confidence interval [CI] was calculated by the exact binomial test, Epi-Info version 6.04b (Centers for Disease Control and Prevention, Atlanta, GA).
† ID = incidence density: episodes per person-years per period (CI calculated by Fisher's exact binomial test, PEPI version 3.0 (Abramson JH, Gablinger P, Salt Lake City, UT)
‡ $P < 0.001$, by chi-square analysis compared to Jan–Mar.
§ $P = 0.035$, by chi-square analysis compared to Jan–Mar.

To determine whether clinical symptoms and hospitalization were associated with specific bacterial pathogens, patients with single infections were analyzed by logistic regression. No association between pathogen isolated and any of the symptoms reported was found except in patients with *Shigella* spp., in whom the risk of having fever (odds ratio [OR] = 4.32, 95% CI = 2.07–9.01, $P < 0.001$) or blood (OR = 5.39, 95% CI = 2.67–10.86, $P < 0.001$) in their stools was significant. Furthermore, individuals with fever and blood in their stools were at significant risk of being diagnosed with shigellosis (OR = 6.20, 95% CI = 1.92–20.01, $P = 0.002$). These results corroborate those of others reporting the isolation of *Shigella* spp. as being associated with fever and blood in the stool.[4,9,16]

Almost half of the patients (139 of 304 with data; 45%) who visited the health post with diarrhea were hospitalized in a local military inpatient facility. Diarrhea patients with shigellosis were significantly more likely to be hospitalized (OR = 2.44, 95% CI = 1.36–4.37, $P = 0.003$) than those without an isolated enteropathogen. Isolation of *P. shigelloides* as a single pathogen approached significance as a risk factor for hospitalization (OR = 7.75, 95% CI = 0.85–372.84, $P = 0.080$).

This study documents a high AR of ADD, predominantly caused by *S. flexneri* 2a resistant to commonly prescribed antibiotics in the region. Although a number of investigations have been performed to determine the incidence and etiology of TD among deployed U.S. troops, local military troops are rarely studied. A study performed between 1993 and 1997 demonstrated that *Shigella* spp. and ETEC were the most common enteropathogens isolated from Israeli troops who acquired ADD while field training during the summer months in Israel.[9] In the Israeli study, the AR for shigellosis was 7.8% (similar to that found in our study), *S. sonnei* and *S. flexneri* were isolated at similar frequencies, and of the *S. flexneri* isolates, serotypes 1 and 6 predominated whereas serotype 2 was less common.[9]

Anti-*Shigella* vaccines have been evaluated in Israeli troops for safety, immunogenicity, and efficacy.[8] The finding that the majority of *S. flexneri* isolates from soldiers at the 5th CIR were serotype 2a has implications for vaccine development because *S. flexneri* vaccines in advanced development are directed towards this serotype. Because the high AR of shigellosis is consistent throughout the year and *S. flexneri* 2a predominates, Peruvian soldiers may be a superior population in which to test the efficacy of *S. flexneri* vaccines. This conclusion, however, is based on findings of just nine months of surveillance and year-to-year variations in the AR and etiology of ADD may occur. Studies are currently underway to identify likely modes of transmission and other potential public health control measures, as well as to assess the capability of this site to support future vaccine clinical trials.

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